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## EXPERIMENTS ON IMMUNIZATION WITH PSEUDO-BLACKLEG PELLETS

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In a previous report<sup>1</sup> it was shown that a certain brand of commercial blackleg vaccine made in the pellet form consisted wholly or in large part of an organism somewhat similar, but distinctly different from *B. chauvei*, and which we called the bacillus of pseudoblackleg, pending the determination of its exact classification. The same peculiarity in the same make of vaccine has recently been observed by Muriel Robertson,<sup>2</sup> and the organism classified by her as *B. oedematis-maligni* Koch. At the time of our first report similar observations of Theobald Smith<sup>3</sup> in 1905 unfortunately were not known to us. Smith states that the strain of blackleg sent him by one manufacturer of blackleg vaccine differed from typical blackleg in gas formula, gas formation from lactose, odor, and action on milk, and concludes: "It may be possible that the one factory uses as blackleg vaccine a wide-spread, spore-forming, pathogenic bacillus, belonging to the group of malignant oedema."

Because so many of these pseudoblackleg pellets were used by the farmers and stock men in the United States, in the vaccination of calves against blackleg, further experiments were undertaken to determine whether these pellets possessed any ability to immunize calves against blackleg.

The plan of the experiment was to vaccinate one group of calves with the commercial pseudoblackleg pellets, and a second group with pellets prepared in our laboratories from blackleg virus secured from naturally occurring cases of blackleg. Both groups of calves were given as nearly as possible the same number of treatments, and the original plan of the experiment called for the pellets prepared from naturally occurring blackleg virus to be of the same virulence to guinea-pigs as the commercial pellets. After two vaccinations all calves were tested for immunity against blackleg by receiving a dose of blackleg virus derived from natural cases of blackleg.

The pellets of the blackleg vaccine under investigation were purchased in the open market, and used within the period of potency as indicated by the expiration date stamped on the package. These pellets will subsequently be

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<sup>1</sup> Jour. Infect. Dis., 1916, 19, p. 408.

<sup>2</sup> Jour. Path. and Bacteriol., 1916, 20, p. 327.

<sup>3</sup> Zeitsch. f. Infektionskr. d. Haust., 1906, 1, p. 26.

referred to as "commercial pellets," and the pellets prepared by us from the blackleg virus as "laboratory pellets." Part of the commercial pellets were of the grade known as "single vaccine," and part were of a stronger grade known as "second double vaccine." The single vaccine was of such virulence that a dose of one pellet killed 90% of the guinea-pigs inoculated. The double vaccine was usually fatal to guinea-pigs in doses of one-half a pellet. This was a higher virulence than the laboratory pellets possessed, although only tissue showing pronounced lesions of blackleg was used in preparing the laboratory pellets, and the period of attenuation was reduced to 80 C. for two hours. The laboratory pellets lost virulence rapidly with age, and therefore were usually made and tested immediately before each treatment. The commercial pellets did not seem to lose virulence during the course of the experiment.

The virus used as a test dose in these experiments was in part obtained from natural cases of blackleg and in part from calves killed by inoculation with such virus. In order to be sure that the natural cases of blackleg were genuine blackleg, in each case the causative organism was isolated and identified as *B. chauvaei*. In addition to the cultural reactions, the virus was further tested against blackleg serum. The serum employed was either that of Foth, or Leclainche and Valle. Guinea-pigs were given an injection of antiblackleg serum and 24 hours later were injected with several lethal doses of the blackleg virus to be tested. If the serum treated guinea-pigs survived the virus injection, and the virus check pigs died, the cultural identification of the active principle of the virus as *B. chauvaei* was considered confirmed.

The size of test dose used in determining immunity of the vaccinated calves was given careful consideration, as it was desired to use a test dose that would produce blackleg in the checks with considerable regularity and without using a larger dose of virus than was necessary. A dose of virus that will kill 50% of the virus check calves is satisfactory, provided one works with a considerable number of animals. Blackleg virus strong enough to kill guinea-pigs in doses of 2 or 3 mg., will kill calves with fair regularity in doses of 1 gm. As the dried virus loses strength rather rapidly, the test dose used in these experiments was not always lethal to guinea-pigs in 3 mg. doses.

The data in regard to the immunization and immunity test on calves follow. This experiment was not done all at once but 7 groups of calves were vaccinated during a period of 9 months, and as nearly as possible half of the calves used at any one time were vaccinated with commercial pellets, and half with laboratory pellets. Each calf received two treatments before being tested for immunity. Sixteen calves were vaccinated with commercial pellets. For their first vaccination 6 of the calves received 1 pellet each of the grade known as double, 5 received 2 pellets each of this grade, while 5 received only 1 pellet of the grade known as single. Measured in terms of lethal doses for a guinea-pig, the pellets given in the first vaccination had a virulence varying between 1 and 4. No ill effects were noted on any of the calves following this vaccination. The second immunization vaccination was given from 11-19 days later. For the second vaccination, the vaccine of the grade known as double was used, and from 2-5 pellets were given, three pellets being the usual dose. The virulence of the vaccine dose used varied between 4 and 10 guinea-pig lethal doses. None of the calves developed any symptoms or disturbance after vaccination. The test for immunity was made about 3 weeks after the second vaccination, though in some instances the time was as short as 16 days, and in one instance as long as 68 days. The time interval did not appear to influence the results. One gram of blackleg virus was used as

the dose in testing for immunity. Of the 16 calves so tested, 8 died of blackleg. As a check on the virus, 8 untreated calves were inoculated with 1 gm. of blackleg virus, and 4 died of blackleg. When this is compared with the results obtained on the calves vaccinated with the commercial pellets, it is evident that no appreciable immunity against blackleg had been given the calves by the commercial pellets. That these calves would have received protection against blackleg had the commercial pellets consisted of attenuated blackleg virus is shown by the results with the laboratory pellets. Eleven calves were vaccinated with laboratory pellets and later tested for immunity against blackleg virus. For the first vaccination with laboratory pellets 6 of the calves received 1 pellet, 3 received  $\frac{1}{2}$  pellet, while 2 received 2 pellets each. The laboratory pellets had a virulence of one lethal dose for a guinea-pig per pellet. No symptoms developed following this vaccination except in the calves receiving 2 pellets. One of these died of vaccination blackleg, and the other was off feed. The second vaccination was given from 11-14 days later, the usual dose being 3 pellets of the same virulence as given. In a few instances 4 or 5 pellets were given. Of the 10 calves vaccinated, 4 showed no symptoms, 5 developed slight swellings at the point of inoculation, and 1 died of vaccination blackleg. The surviving 9 calves of this group were tested for immunity from 12-33 days later by inoculation with 1 gm. of blackleg virus. All withstood the immunity test.

It should be cautioned that the immunization of calves in actual practice cannot safely be carried out with such a strong vaccine as was used in this experiment. It is of interest to note that at the same time this experiment was carried out, calves on farms in the vicinity of Manhattan were vaccinated with laboratory pellets prepared as for immunization in this experiment, but not quite so virulent. Forty-one calves were vaccinated with 1 pellet having a virulence of from  $\frac{1}{2}$ -1 guinea-pig lethal dose. Of the 41 calves, 4 died from vaccination blackleg. The susceptibility of calves varies so greatly that a large margin of safety must be maintained when administering spore containing vaccines.

#### CONCLUSIONS

One half of the calves treated with pseudoblockleg commercial pellets succumbed to a 1 gm. test dose of blackleg virus.

All calves treated with true blackleg laboratory pellets were immune against a 1 gm. test dose of blackleg virus.

One half of the nontreated calves succumbed to a 1 gm. dose of blackleg virus.

The pseudoblockleg commercial pellets did not immunize calves against blackleg under conditions in which true blackleg vaccine produced immunity without exception.